

REPORT DOCUMENTATION PAGE

2001 Dec Form Approved
OMB NO. 0704-0188

Public Reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comment regarding this burden estimates or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188) Washington, DC 20503.

1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED	
	12/15/01	Final Progress Report 6/1/01-11/30/01	
4. TITLE AND SUBTITLE Characterization and Optimization of Novel Nanostructured Metallic Substrates for Surface Enhanced Raman Spectroscopy		5. FUNDING NUMBERS DAAD19-01-1-0576	
6. AUTHOR(S) Abraham M. Lenhoff, Orlin D. Velev and Eric W. Kaler		8. PERFORMING ORGANIZATION REPORT NUMBER 42709.1-CH-11	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Department of Chemical Engineering University of Delaware Newark, DE 19716			
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U. S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211		10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.			
12 a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.		12 b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) Surface-enhanced Raman spectroscopy (SERS) is a highly sensitive technique for quantifying trace amounts of analyte adsorbed at a roughened metal surface. We have shown that novel gold nanostructured films, simply fabricated using gold nanoparticles and latex microspheres, can be used as highly sensitive SERS substrates. The films are templated by layers of colloidal crystals and display long ranged ordered regions, and porosity on two length scales that results in high surface area. By integrating these substrates into a flow chamber we have obtained consistent reproducible data and demonstrated the quantitative detection of sodium cyanide in the concentration range from 5 to 500 ppb, with detection significantly enhanced by lowering the pH after cyanide adsorption. We have found that the optimum size of the latex microspheres is approximately equal to the wavelength of the laser. Finally, the SERS activity of the gold substrates coated with silver nanoparticles was investigated using methyl phosphonic acid (MPA). Detection of MPA was dependent on the time between substrate coating and the SERS measurement. These studies show that novel materials formed by simple wet chemistry techniques can be used efficiently in practical devices for the detection of chemical agents.			
14. SUBJECT TERMS		15. NUMBER OF PAGES 17	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OR REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION ON THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL

20020201 117
JM

MEMORANDUM OF TRANSMITTAL

U.S. Army Research Office
ATTN: AMSRL-RO-BI (TR)
P.O. Box 12211
Research Triangle Park, NC 27709-2211

Reprint (Orig + 2 copies) Technical Report (Orig + 2 copies)
 Manuscript (1 copy) Final Progress Report (Orig + 2 copies)
 Related Materials, Abstracts, Theses (1 copy)

CONTRACT/GRANT NUMBER: DAAD19-01-1-0576

REPORT TITLE:

Characterization and Optimization of Novel Nanostructured Metallic Substrates for Sustrates for Surface Enhanced Raman Spectroscopy

is forwarded for your information.

SUBMITTED FOR PUBLICATION TO (applicable only if report is manuscript):

Sincerely,

Abraham M. Lenhoff
Professor

Enclosure 3

Characterization and Optimization of Novel Nanostructured Metallic Substrates for Surface Enhanced Raman Spectroscopy

ARO Final Report Submitted to

Dr. Stephen Lee

U.S. Army Research Office

P.O.Box 12211, Research Triangle Park,

NC 27709-2211

By

Orlin D. Velev, Eric W. Kaler and

Abraham M. Lenhoff

Department of Chemical Engineering

University of Delaware

December 2001

ABSTRACT

Surface-enhanced Raman spectroscopy (SERS) is a highly sensitive technique for quantifying trace amounts of analyte adsorbed at a roughened metal surface. Many techniques, including electrochemical etching and e-beam lithography, have been previously used to produce roughened metallic surfaces. In this report we demonstrate that novel gold nanostructured films, which are simply fabricated using gold nanoparticles and latex microspheres, can be used as highly sensitive SERS substrates. The gold films are templated by layers of colloidal crystals and display long ranged ordered regions. These films are porous on two length scales and, therefore, possess a high surface area. The SERS activity of the substrates was characterized using sodium cyanide as a model compound. By integrating these substrates into a flow chamber we have obtained consistent repeatable data and demonstrated the quantitative detection of sodium cyanide in the concentration region from 5 to 500 ppb. These data reveal that cyanide detection can be significantly enhanced by lowering the pH after cyanide adsorption, likely indicating a conformational change of the bound cyanide. We have found that the optimum size of the latex microspheres is approximately equal to the wavelength of the laser. Finally, the SERS activity of the gold substrates coated with silver nanoparticles was investigated using methyl phosphonic acid (MPA). We found that detection of MPA was dependent on the time between coating the substrate with silver nanoparticles and measuring the SERS spectra. These studies show that novel materials formed by simple wet chemistry techniques can be used efficiently in practical devices for the detection of chemical agents.

INTRODUCTION

It has been long recognized that the key to making SERS a routine detection method for analytical methods is to develop substrates that are highly sensitive, selective, reproducible and inexpensive. Methods which produce a roughened metallic surface, such as electrochemical techniques,¹ metallic nanoparticles adsorbed on a surface,² vapor deposition of metal onto a roughened surface,³ and microfabrication of patterned metallic structures⁴ cannot meet all of these requirements. Specifically, electrochemical roughening has the disadvantage of poor reproducibility, and microfabrication has the disadvantage of being expensive and time consuming.

The use of metallic nanoparticles on a solid substrate is one of the most promising and simple methods of forming SERS substrates. Previous work has shown show that metallic nanoparticles adsorbed on a glass surface using either biospecific or metal-affinity interactions,⁵ could serve as SERS substrates, however these structures lack advanced features, such as periodicity, which are present in microfabricated structures.^{5, 6} Also, all previously proposed methods of making SERS substrates lack the ability for making three dimensional ordered structures, which may have a higher SERS activity.

We have recently introduced a method of forming three dimensional nanostructured metallic films on microscope slides.^{7, 8} The structures are formed by using colloid crystals to template gold nanoparticles into multicrystalline films. Gold and latex particles are mixed together and slowly coated on the surface of a substrate. As the solvent evaporates from the film colloidal crystals from the latex microspheres form initially, and, as the liquid recedes into the interstitial voids of the colloidal crystals, the gold nanoparticles are

packed together around the latex crystal template. The latex spheres can then be removed by dissolution in an organic solvent, leaving a three dimensional array of gold nanoparticles arranged into an ordered pattern. The advantages of this method are the rapid, simple and inexpensive fabrication of SERS substrates with advanced features including periodicity. Previously we have shown that these structures are highly sensitive SERS substrates for the detection of BPE.^{7,8}

In this report we describe how to form novel SERS substrates and how these substrates can be integrated into an on-line flow chamber, which can be used to study the effect of solution conditions and additives on the detection performance of sodium cyanide. The overarching goal of this work is to develop a precise and robust procedure for characterization of the substrates in flow devices and understand the factors that enhance or impede the sensitivity of practical devices based on such microstructured substrates. A shortcoming of many previous studies is that the sample to be detected was applied to the substrate either by drying the sample on the substrate or encapsulating the sample in a stagnant solution. Both of these methods must assume that the substrates are uniform in structure and that the analyte is evenly dried on the surface, which is likely not true, so the effects of solution conditions and analyte concentration may not be evaluated properly. We demonstrate a simple method for sampling the same location repeatedly in a flow chamber, which allows the complex SERS dependence of surface structure to be decoupled from the adsorption equilibria and surface properties of cyanide bound to gold. The use of flow chamber continuously sampling a flux of liquid opens the way to the practical application of SERS detectors in chemical defense for military and civilian purposes.

EXPERIMENTAL

Materials and Substrate Preparation. The gold particles were prepared by the standard procedures reported earlier.^{9, 10} The gold nanoparticles were 25 nm in diameter. These particles were concentrated using 5 kD MWCO centrifuge filters, which concentrated the solution approximately 100 times. The gold solution was then further centrifuged (in the absence of a filter) to concentrate the solution another order of magnitude. The final gold concentration was typically 1-5 wt%.

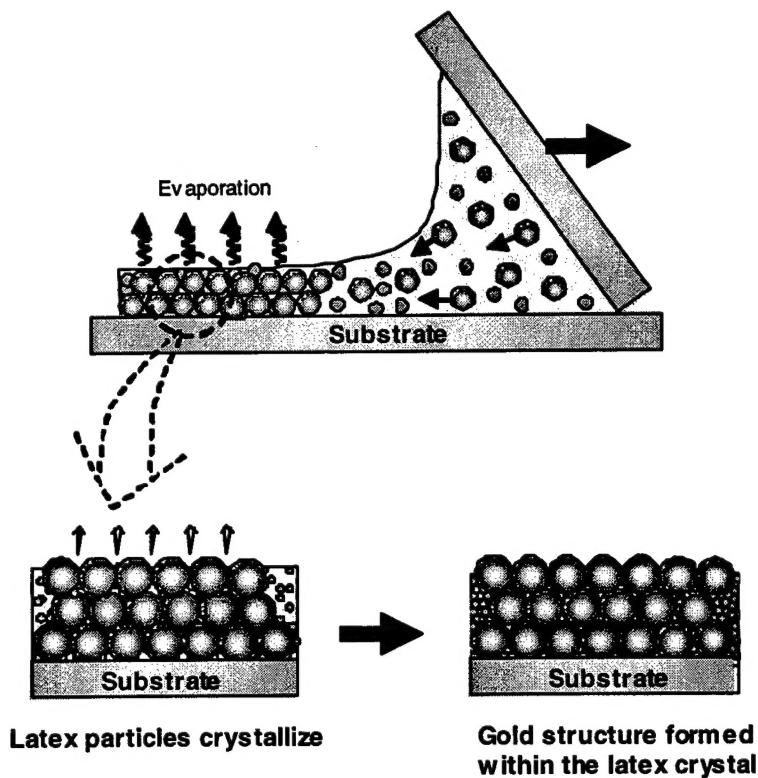


Figure 1. Schematic diagram of the assembly method used in this work for forming macroporous/mesoporous gold films.

Negatively charged polystyrene microspheres (650 nm) were diluted 10 times in deionized water, Tween 20 was then added to further stabilize the latex particles (final conc. of 0.0002 wt. %) and then the particles were settled by centrifugation. The final concentration of the microspheres was ca. 20 wt%. Approximately 8 μl of concentrated

gold and 4 μ l of concentrated latex were mixed and spread onto a glass slide, as shown in Figure 1. The top plate was attached to a linear motor, and was translated horizontally at $\approx 2 \mu\text{m/s}$. The relative humidity strongly affected the degree of ordering and the drying time, although the latex/gold films were typically dry within 45 minutes. The substrates were then washed in toluene to remove the latex particles, and finally they were treated with dimethylchlorosilane to promote the adhesion of the gold structures to the glass substrates.

On-line Measurements and Instrumentation. Figure 2 shows how the nanostructured films were integrated into a flow chamber that we designed for on-line detection. A small microscope chamber (170 μl) was clamped to the substrate using two steel square plates, each with a circular opening to optically access the gold film. Teflon tubing (0.08 cm diameter at the inlet, 0.16 cm at the outlet) was then connected to the two microscope chamber ports and a peristaltic pump was used to pass the sample through the flow chamber at 0.4 ml/min. Teflon grease was used to seal the port opening to prevent water leakage at these connections.

The chamber was then placed inside the Raman detection system. The Raman instrument consists of an EIC Laboratories, Inc. echelle spectrograph Model NIR700 (approximately 1 cm^{-1} resolution), diode laser operating at 785 nm, and EIC RamanProbeTM fiber optic Raman probe. Spectra were collected and processed by the EIC2000 software package. The laser power was limited to approximately 30 mW to avoid damaging the substrate, and the sample collection time was 60 sec. To avoid interference

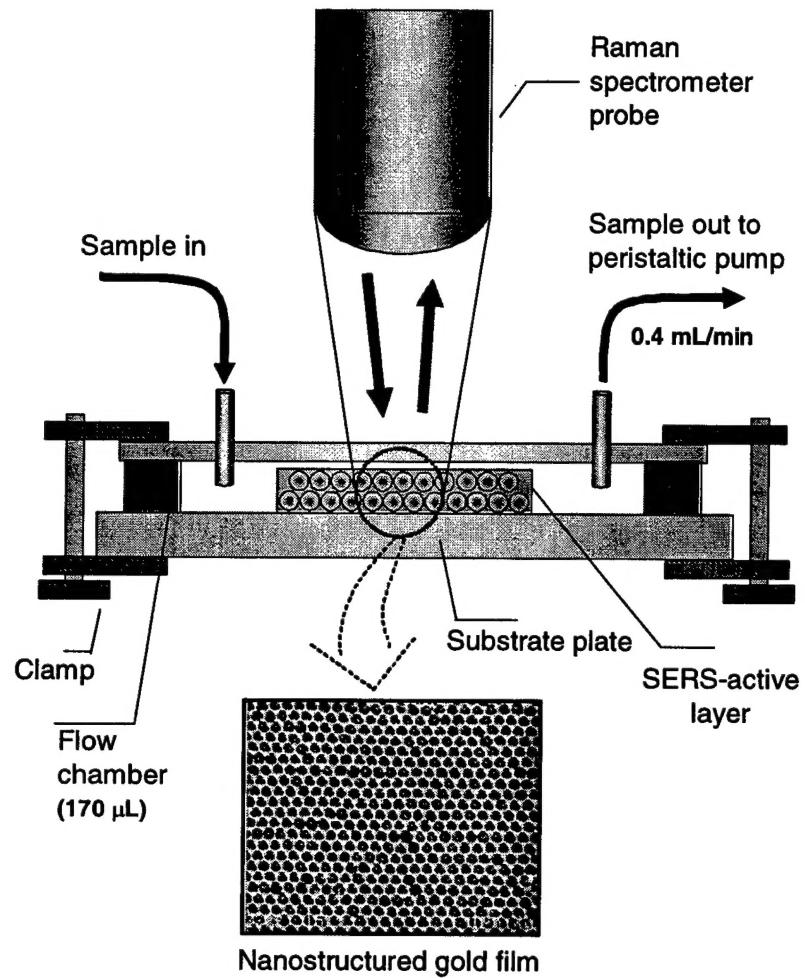


Figure 2. Schematic diagram of the Raman flow chamber integrated with the nanostructured gold films.

in the spectra by ambient light, the flow cell was placed on an X-Y translation stage in a dark box containing the fiber-optic probe. The translation stage was used to position the substrate at the focus of the RamanProbeTM, and the position of the beam spot on the substrate was monitored with a CCD camera.

RESULTS AND DISCUSSION

Substrate treatment and experimental methodology. Since gold is generally hydrophobic and glass is hydrophilic, the gold structured films were initially not well adhered to the microscope slides. We have solved this problem by treating the substrates with dimethylchlorosilane, which made the microscope slides hydrophobic and enhanced the adhesion of the gold to the glass substrates. Afterward, the substrates were mechanically stable in flowing water stream and could be used for extended periods of time without the metal peeling off the glass substrate. This good substrate stability allowed performing repeated experiments on the same position of the substrate at various solution conditions, and thus separating the effects of the substrates from the effects of the medium conditions.

Effect of pH on the signal intensity. A major finding was that the Raman signal of cyanide was dependent on the pH of the solution used to adsorb cyanide and the pH of the solution used after adsorption during detection. The pKa of sodium cyanide is approximately 9.5. At neutral pH, cyanide does not adsorb on the gold, which likely is due to its being in a non-ionized state. At high pH (~13), cyanide adsorbs on gold readily, as shown in Figure 3A. Surprisingly, the Raman signal for cyanide can at this stage be enhanced by reducing the pH of the solution (without adding more cyanide) by flowing dilute nitric acid (0.01 M HNO₃) through the flow cell, as shown in Figure 3B. This effect was reversible since the pH could be increased with 0.1 M NaOH (again without any further addition of cyanide), which gave the same spectra as before (Fig. 3C). Subsequent treatment of the substrate with dilute nitric acid gave similar spectra as before (Fig. 3D). It

should also be noted that the cyanide peak shifted from 2130 cm^{-1} at neutral pH to 2140 cm^{-1} at basic pH.

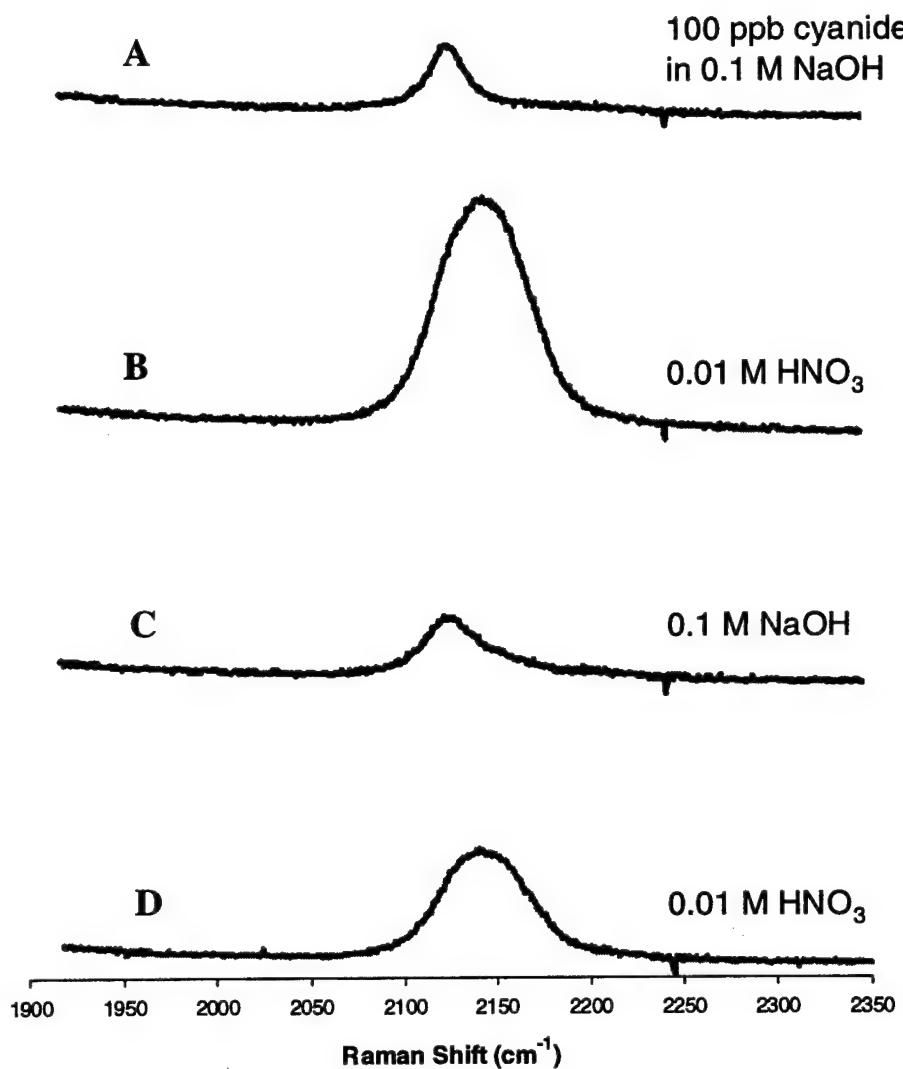


Figure 3. Effect of the solution pH on the detection of 100 ppb sodium cyanide.

The origin of the pH dependence of the Raman signal appears to be due to a conformational change of adsorbed cyanide on the gold surface. The ionization state of cyanide in solution changes between neutral and very basic pH, which also supports that a conformational change may occur. It has been shown previously that the bonding

mechanism for cyanide to gold in an aqueous environment is largely ionic,¹¹ in contrast to carbon monoxide, which bonds to gold in a mostly covalent manner. It has also been shown the adsorption energies of cyanide on nickel varies by only a few percent, indicating that a change in orientation may be possible due to a change in the pH.^{12, 13} Finally, the solution and surface bound properties of cyanide seem to be quite different, since although cyanide molecules are not adsorbed on the gold particles at neutral pH, they remain adsorbed at neutral pH once the molecules have been bound at high pH.

Effect of cyanide concentration. We have characterized the linear range of our SERS substrates for the detection of sodium cyanide, which is shown in Figure 4. The normalized peak area, which is proportional to the amount adsorbed, versus the log of the cyanide concentration is linear, which indicates a Langmuir type isotherm. Others have shown that a monolayer surface coverage of cyanide on gold corresponds to a solution concentration of 500 ppb cyanide,¹⁴ which agrees well with our results. Also above 500 ppb, we observed a reduction in cyanide signal, likely indicating that the excess cyanide caused dissolution of the gold. Further, although it has been reported that cyanide adsorbed on a silver electrode gave a linear dependence between peak area and cyanide concentration on a log-log plot,¹⁵ this result likely is not in contradiction with our results since our data is approximately linear on a log-log plot. The practical lower limit of detection achieved in this work was 5 ppb sodium cyanide.

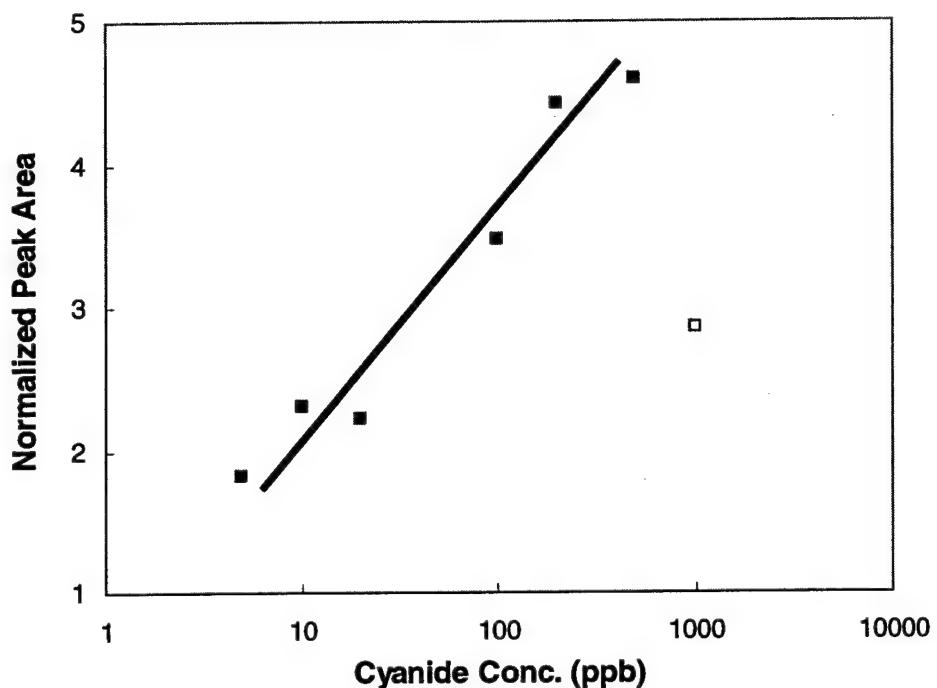


Figure 4. Effect of cyanide concentration on the Raman signal for sodium cyanide.

Effect of Size of Latex Particles. We studied the effect of the size of the latex particles on the measured peak area for 100 ppb cyanide in 0.1 M NaOH, which is shown in Figure 5. Interestingly we observed a maximum in the Raman signal at a latex microsphere size (720 nm) that roughly corresponded to the wavelength of the laser (785 nm). This behavior suggests that light interacts with the structured film in a resonance manner, which can be compared in principle to photonic crystals that exhibit a maximum reflectance at wavelengths of light corresponding to their band gap. The cavities of the structured material are comparable in size to the wavelength of light and this acts as an array of chambers resonating at a specific frequency. Similar behaviour has been observed previously with microfabricated substrates, however our method allows very facile and easy tuning of the resonance frequency via the diameter of the latex templates. Precisely matching the size of the latex particles may lead to further Raman enhancement.

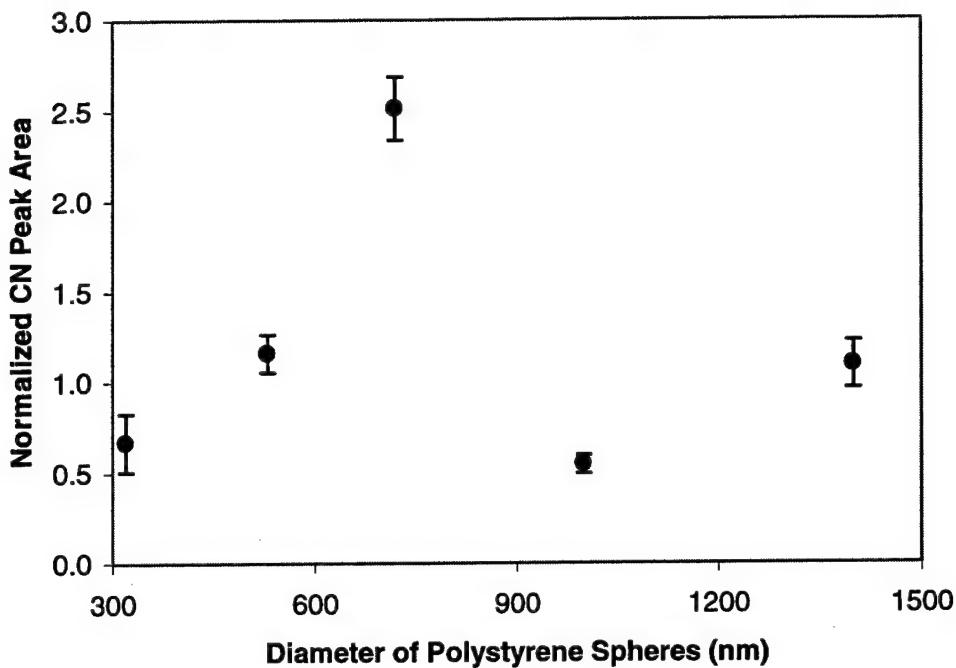


Figure 5. Effect of the size of the latex spheres on the measured Raman peak area for 100 ppb cyanide in 0.1 M NaOH at 2125 cm^{-1} .

Effect of Film Thickness. The thickness of the films could be varied typically between one to two layers of latex particles thick as shown in Figure 6. To study the effect of film thickness on the detection of cyanide, it was necessary to develop an experimental procedure to identify the location on the substrate that was sampled by the laser. We have done this by burning a hole in the substrate by raising the laser power to much higher levels than that used for the actual sampling. Afterward, we could identify the location of the hole by SEM and determine whether the film was a monolayer or bilayer. In Figure 6, both images show an edge of a hole that was created by the laser.

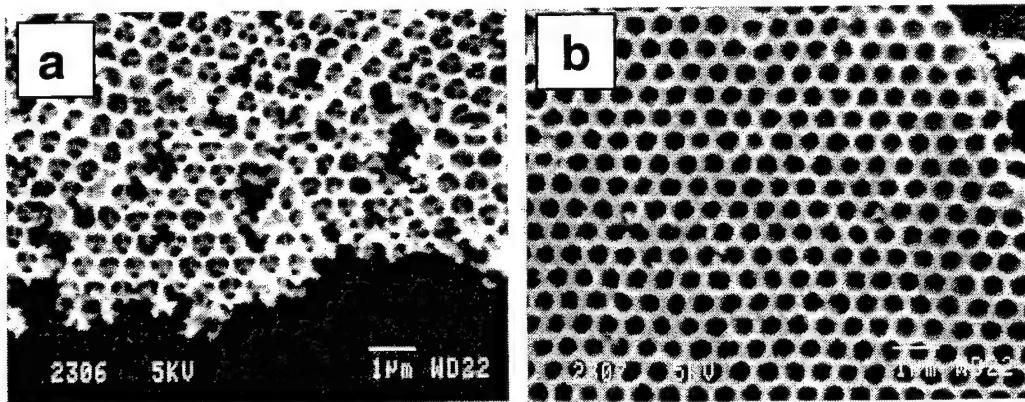


Figure 6. Typical (a) bilayer and (b) monolayer structures which were formed using the deposition method described in this work.

Figure 7 shows Raman spectra obtained by structures that were determined to be monolayer and bilayer. Further data is required to confirm these results, but it appears that increasing thickness leads to increasing enhancement, which is likely due to the increased gold content and substrate area in the pores per scattering cross section. However, it is unlikely that further increase the film thickness past a bilayer or trilayer will cause a concomitant increase in the Raman signal. We will look for optimization of the gold thickness in our future studies.

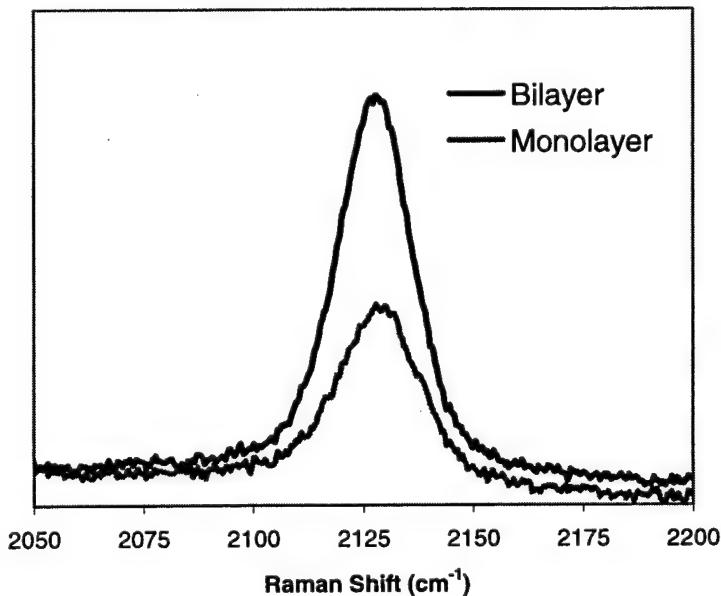


Figure 7. Raman spectra for 100 ppb cyanide adsorbed initially in 0.1 M NaOH, and then measured in water.

Preliminary Studies of Nontemplated Porous Films. We also examined the possibility of preparing porous gold films without the use of latex particles in order to understand the role of substrate porosity and structure for the intensity of the SERS spectra. The deposition procedure was similar to that described above, but instead we used only a concentrated gold suspension (without latex microspheres), and dried a small amount (~10 µL) of the gold directly on a glass microscope slide. The resulting gold films were thick and appeared as a smooth gold mirror. Further inspection of the gold films by SEM revealed that two different types of structures could be prepared by this procedure (Figure 8). The film in Figure 8a has macropores that are approximately a half micron in size, which is similar to the size of the latex particles used in the template films. However the film in Figure 8b, prepared by the same procedure but with a different batch of gold, shows no macropores. We speculate that the difference between the two films is due to a differing degree of aggregation of the nanoparticles before the gold film was formed.

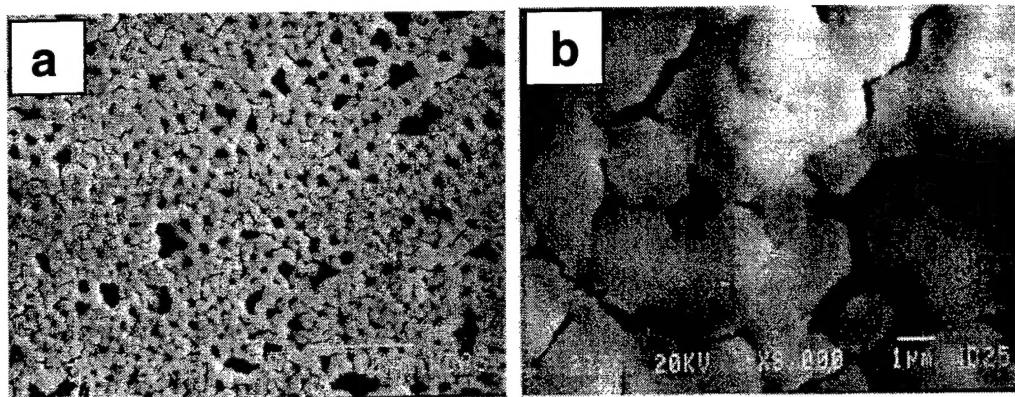


Figure 8. Gold nanostructured films formed without latex templates: (a) macroporous gold layer likely formed from aggregated gold, (b) gold layer without macropores likely formed from unaggregated gold.

Preliminary characterization of these films by Raman spectroscopy showed significant differences. The porous film showed enhancements greater than that obtained with the templated materials, likely due to the larger amount of gold per scattering cross section. However, the nonporous films were unable to detect 100 ppb cyanide, suggesting that materials without pores do not have the proper surface topology for the sensitive SERS detection of cyanide with gold colloids.

Effect of silver substrates on the detection of MPA. A silver enhancing kit (BBInternational) was used to modify the surface of the gold substrate in order to detect MPA. Enhancement of the substrate involves reduction of silver from one solution (the enhancer) by another (the initiator) in the presence of the gold particles. This process may be sensitive to light and oxygen, resulting in degradation of the substrate coating. We found that a slide prepared two weeks prior to MPA exposure showed no sign of MPA detection by SERS, despite being stored in the dark. However, when a fresh layer of silver particles was deposited on a substrate previously coated with silver it was possible to detect the presence of 1 ppt MPA in water. This worked better than depositing the silver directly onto an untreated gold substrate and measuring for MPA detection immediately. This area requires further investigation in our future studies.

CONCLUSIONS

We have studied the SERS response of gold nanostructured substrates to sodium cyanide in a flow chamber. Quantitative detection of cyanide from 5 to 500 ppb was demonstrated at neutral to basic pH. The Raman signal for cyanide could be enhanced after adsorbing cyanide at high pH by reducing the pH with dilute nitric acid, which is likely

due to a conformational change of the bound cyanide on gold. We have also shown preliminary results on determining the optimum thickness and periodicity for the detection of sodium cyanide. The data presented in this paper illustrates a method to optimize the design of continuous on-line devices for cyanide detection in water. We have been able to perform preliminary experiments to detect phosphonates on substrates made from silver nanoparticles. The data demonstrate the applicability of our substrates and the developed flow chamber in army and civilian devices for chemical detection.

REFERENCES

1. N. Taranenko, J.P. Alarie, D.L. Stokes and T. Vo-Dinh, "Surface-enhanced Raman detection of nerve agent simulant (DMMP and DIMP) vapor on electrochemically prepared silver oxide substrates", *J. Raman Spectrosc.* **27**, 379, 1996.
2. R.G. Freeman, K.C. Graber, K.J. Allison, R.M. Bright, J.A. Davis, A.P. Guthrie, M.B. Hommer, M.A. Jackson, P.C. Smith, D.G. Walter and M.J. Natan, "Self-assembled metal colloid monolayers: an approach to SERS substrates", *Science* **267**, 1629, 1995.
3. R.L. Moody, T. Vo-Dinh and W.H. Fletcher, "Investigation of experimental parameters for surface-enhanced Raman-scattering (SERS) using silver-coated microsphere substrates", *Appl. Spectrosc.* **41**, 966, 1987.
4. M. Kahl, E. Voges, S. Kostrewa, C. Viets and W. Hill, "Periodically structured metallic substrates for SERS", *Sensor Actuat. A-Phys.* **51**, 285, 1998.
5. K.C. Graber, R.G. Freeman, M.B. Hommer and M.J. Natan, "Preparation and characterization of Au colloid monolayers", *Anal. Chem.* **67**, 735, 1995.
6. J.C. Hulteen and R.P. Van Duyne, "Nanosphere lithography: A materials general fabrication process for periodic particle array surfaces", *J. Vac. Sci. Technol. A* **13**, 1553, 1995.
7. P.M. Tessier, O.D. Velev, A.T. Kalambur, A.M. Lenhoff, J.F. Rabolt and E.W. Kaler, "Assembly of gold nanostructured films templated by colloidal crystals and use in surface-enhanced Raman spectroscopy", *J. Am. Chem. Soc.* **122**, 9554, 2000.
8. P.M. Tessier, O.D. Velev, A.T. Kalambur, A.M. Lenhoff, J.F. Rabolt and E.W. Kaler, "Structured metallic films for optical and spectroscopic applications via colloidal crystal templating", *Adv. Mater.* **13**, 396, 2001.
9. J.W. Slot and H.J. Geuze, "Sizing of protein A-colloidal gold probes for immunoelectron microscopy", *J. Cell Biol.* **90**, 533, 1981.
10. J.W. Slot and H.J. Geuze, "A new method of preparing gold probes for multiple-labeling cytochemistry", *Eur. J. Cell Biol.* **38**, 87, 1985.
11. M.R. Philpott, P.S. Bagus, C.J. Nelin and H. Seki, "Vibrational and electronic properties of cyanide adsorbed on metal electrodes", *J. Electron Spectrosc.* **45**, 169, 1987.
12. H. Yang, "Ab initio embedding studies of chemisorption on metal surfaces - interaction of small C-N containing molecules with Ni(111)", *J. Mol. Catal. A-Chem.* **119**, 425, 1997.
13. H. Yang and J.L. Whitten, "Energetics of adsorption and coadsorption of CN and O on Ni(100)", *J. Chem. Phys.* **107**, 8518, 1997.
14. P. Gao and M.J. Weaver, "Vibrational coupling effects for cyanide and aromatic adsorbates at gold electrodes - a comparative study using surface Raman and infrared spectroscopies", *J. Phys. Chem.-US* **93**, 6205, 1989.
15. R.D. Shelton, J.W. Haas and E.A. Wachter, "Surface-enhanced Raman detection of aqueous cyanide", *Appl. Spectrosc.* **48**, 1007, 1994.